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ORIGINAL PAPER

Genetic diversity of N-fixing and plant growth-promoting bacterial community in different sugarcane genotypes, association habitat and phenological phase of the crop

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Abstract

This study aimed to evaluate the genetic diversity of bacterial community associated to different sugarcane genotypes, association habitat and phenological phase of the culture, as well as to isolate, to identify and to characterize your potential for plant growth-promoting. Root and rhizospheric soil samples from RB 92579 and RB 867515 varieties were collected at 120 and 300 days after regrowth (DAR). The diversity of bacterial was evaluated through of the 16S rRNA and *nif*H genes. We found greater genetic diversity in the root endophytic habitat at 120 DAR. We identify the genera *Burkholderia* sp., *Pantoea* sp., *Erwinia* sp., *Stenotrophomonas* sp., *Enterobacter* sp. and *Pseudomonas* sp. The genera *Bacillus* sp. and *Dyella* sp. were only identified in the variety RB 92579. We found indices above 50% for biological nitrogen fixation, production of indole acetic acid and phosphate solubilization, showing that the use of these bacteria in biotechnological products is very promising.

Keywords BOX-PCR · DGGE · Biological N fixation · Production of indole acetic acid · Phosphate solubilization

Introduction

Sugarcane nitrogen (N) demand in the second crop cycle (ratoon-cane) increases by an average of 50% in relation to the first crop cycle (plant-cane) (Sattolo et al. 2017). The use of chemical N fertilizer to meet this elevated demand increases the probability of N loss in the soil–plant-atmosphere system, increasing its environmental impact (Kyllmar et al. 2014) and creating a need for studies examining alternatives that may reduce the use of N fertilizers, such as biological N fixation (BNF).

BNF can be performed in sugarcane by several bacterial genera, such as: *Beijerinkia* sp. (Dobereiner and Ruschel 1958), *Citrobacter* sp., *Brevibacillus* sp., *Curtobacterium* sp. (Magnani et al. 2010), *Asaia sp.*, *Ochrobactrum* sp., *Rhizobium*, *Stenotrophomonas* sp., *Acinitobacter* sp., *Klebisiella* sp., *Azospirillum* sp., *Azorhizobium* sp., *Xanthobacter* sp. (Beneduzi et al. 2013), *Burkholderia* sp., *Enterobacter* sp., *Pantoea* sp. and *Pseudomonas* sp. (Silva et al. 2016). However, studies of genetic diversity as a function of the habitat of the bacterial association and the phenological phase of the cultivation of commercial sugarcane genotypes are few and necessary. These studies can identify promising strains, which in addition to N fixation can be promoters of plant growth.

N-fixing bacteria found in association with non-leguminous plant species may in habit different habitats such as: rhizosphere, the surface or the interior of plant tissues,

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colonizing different organs of the plant. These bacteria are found in greater density in the rhizosphere and at the surface of the roots. When they colonize the endophytic tissues, they are found in greater proportion within the roots, decreasing progressively towards the aerial part (Compant et al. 2019). Therefore, evaluating where the highest bacterial density occurs in different commercial sugarcane genotypes can help the application of future technological bioproducts based on N-fixing and plant growth-promoting bacteria.

N-fixing bacteria that colonize sugarcane have a great number of functions, such as: production of indole acetic acid (IAA); production of siderophores; phosphate solubilization (PS); production of 1-aminocyclopropane-1-carboxylic acid; *quorum-sensing* (QS) molecules production; endonuclease production; protease production; laccase production; biocontrol; and synthesizing cytokinin and gibberellin, among other amino acids (Vejan et al. 2016). N-fixing bacteria performs these functions related to the plant growth-promotion due to its high genetic diversity, which can be altered when submitted to adverse conditions and may form different structural groups (Cordero et al. 2016; Oliveira et al. 2017).

Some studies in the literature have shown the formation of different structural groups in the bacterial community. Yeoh et al. (2016) and Li et al. (2016) evaluating the diversity of the bacterial community sampled in the rhizosphere and rhizomes of plants of *Micanthus giganteus* through the 16S rRNA and *nif*H genes verified the formation of distinct structural groups in these different habitats of plant/bacteria association. Leite et al. (2014) evaluated the effect of salinity on the bacterial community associated with sugarcane and reported that there was change in the formation of structural groups of rhizosphere and root endophytic strains. Rodríguez-Blanco et al. (2015) evaluating the *nif*H gene of diazotrophic bacteria inoculated in two genotypes of corn, both with and without N fertilization observed the formation of four distinct structural groups.

Thus, changes are observed in the structural groups of the bacterial community in the different habitats of the association and genotypes. However, there are no reports of bacterial changes in structural groups within the same cultivation cycle, especially in sugarcane. It is important to know the genetic diversity and the ability of the bacterial community associated with sugarcane to carry out BNF and promote plant growth in different phenological stages of cultivation to support the management of the application of future technological bioproducts, identifying the best time of application. Urquiaga et al. (2012) reported that this ability to carry out BNF of bacteria in sugarcane increased with the cultivation cycles and Santos et al. (2019) showed that nitrogenase activity was maximum at 100 days after the establishment of sugarcane plants. This highlights the importance of assessing genetic diversity in different phenological stages of sugarcane cultivation.

We believe that the genetic structure of the N-fixing bacterial community associated with sugarcane changes according to association habitat independent of variety, because the plant/bacterial association is not specific. It may be that bacterial diversity decreases during cultivation and is lower in the maturation stage of the crop. However, this N-fixing bacterial community has the potential to plant growth-promotion, regardless of variety, association habitat or phenological phase of culture. It may be that changes in the bacterial community structure influences the plant growth-promotion, promoting to a greater effect depending on the variety, association habitat and/or phenological phase the sugarcane.

Studies evaluating these relationships between the N-fixing bacterial communities associated with sugarcane in its genotypic aspects, infestation sites and phenological phase of cultivation can contribute to an increased efficiency of the plant/bacteria association and promote a reduction in the use of N chemical fertilizers, reducing its harmful effects on the environment. Similarly, studies that identify and characterize the plant growth-promotion potential of microorganisms may be useful to broaden our knowledge of these relationships.

Thus, this study aimed to evaluate the genetic diversity of bacterial community associated to different sugarcane genotypes, association habitat and phenological phase of the culture, as well as to isolate, to identify and to characterize your potential for plant growth-promoting.

Materials and methods

Site characterization

The study was carried out at the Carpina Sugarcane Experimentation Station in the sugarcane-producing region of the Pernambuco State in Northeast Brazil, located at geographical coordinates 7°51′03′′ south latitude and 35°15′17′′ west longitude at an altitude of 184 m. The predominant climate in the region is type As according to the Köppen classification (Alvares et al. 2013), rainy tropical with dry summer, average annual precipitation of 1100 mm and an average annual temperature of 24.2 °C (Beltrão et al. 2005).

The cultivation soil of the sugarcane varieties was classified by the Brazilian Soil Classification System as Argissolo Amarelo distrocoeso (Santos et al. 2013), corresponding to Ultisol (Soil Survey Staff 2014). It is a common soil in the Brazilian Northeast which is predominant in Pernambuco and generally used for growing sugarcane.

Collection of root samples and rhizospheric soil

Samples of both sugarcane roots and rhizospheric soil were collected in April and October 2010 from commercial planting areas of RB 92579 and RB 867515 varieties at 120 and 300 days after regrowth (DAR) of ratoon-cane. The maximum tillering phenological phase occurs at 120 DAR and maturation of stalks with high levels of sucrose accumulation occurs at 300 DAR (Bonnett 2013). RB 92579 and RB 867515 sugarcane varieties were utilized in the study because of their extensive cultivation, both in the Northeast and throughout Brazil (Simões Neto et al. 2005). Fertilization of ratoon-cane corresponded to the application of 100 kg ha^{-1} of N and 100 kg ha^{-1} of K, right after harvesting the previous cycle. The sources of N and K used were ammonium sulfate and potassium chloride, respectively. Samples of rhizospheric soil and roots were collected of five randomly selected plants of each variety from each phenological phase of the cultivation. Rhizospheric soil samples were collected to a depth of 20 cm. After root collection, the excess soil was withdrawn through circular movements and the remaining soil in direct contact with the roots was collected (Oliveira et al. 2017).

Samples were stored in ice styrofoam, identified and transported to the laboratory, where part of the rhizospheric soil and roots were used for isolation procedures and tests to plant growth-promoting and identification, while another part was separated and stored in microtubes that were then placed in a freezer at − 18 °C for subsequent DNA extraction and total bacterial community diversity analysis.

Genetic diversity total bacterial community

DNA extraction from root and rhizospheric soil samples was performed using the Power Soil DNA kit (MoBio, USA). The integrity and quality of the DNAs were verified by 1% (w/v) agarose gel electrophoresis in 1 × TAE buffer combined with a GeneRuler 100 bp DNA Ladder (Thermo Scientific) molecular weight marker and then observed under ultraviolet light and photodocumented.

16S rRNA Gene

Two *Polymerase Chain Reactions* (PCR) were performed to analyze *Denaturing Gradient Gel Electrophoresis* (DGGE) of the total bacterial community in the rhizospheric soil. The 1st PCR was prepared for a final volume of 50 µL containing: about 1.0 µL of total soil DNA (10 ng); 0.1 µL (0.2 µM) of each specific *primer* for the 16S rDNA gene [338F-GC (5′-GC-clampACTCCTACGGGAGGCAGCAG-3′)] and R518 (′5-ATTACCGCGGCTGCTGG-′3) (Ovreas et al. 1997); 4.0 µL of each 0.2 mM dNTPs; 5.0 µL of 10X Taq buffer; 7.5 µL of $MgCl_2$ (1.5 mM); and 0.5 µL of the Taq DNA polymerase enzyme (Fermentas) (2.5U), with ultrapure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 30 cycles of 1 min at 95 °C; 1 min at 92 °C; 1 min at 55 °C; 1 min at 72 °C and 10 min at 72 °C. The 2nd PCR was also prepared for a final volume of 50 µM, containing: 1 µL of the product from the 1st PCR; 0.2 µL (2 µM) of each *primer* [F968-GC (5′-AACGCGAAGAACCT TAC-3′)] (Nübel et al. 1996) and R1387 (5′-GCCCGGGAACGTATT CACCG-3′) (Heuer et al. 1997); 4.0 µL 2.5 mM dNTPs; 5.0 µL 25 mM $MgCl_2$; 5.0 µL 10 × Taq buffer; 0.5 µL formamide; and 0.5 µL of the Taq DNA polymerase enzyme (Fermentas), with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 4 min at 94 °C; 30 cycles of 1 min at 94 °C; 1 min at 56 °C; 2 min at 72 °C and 10 min at 72 °C.

Two PCRs were also performed for the DGGE analysis of the total root endophytic bacterial community. The 1st PCR was prepared for a final volume of 50 µL, containing: about 20 ng of total root DNA; 1.0 µL (0.1 µM) of each *primer* [799f (5′-AACMGGATTAGATACCCKG)] (Chelius and Triplett 2001) and 1492r (5′-GGYTACCTTGTTACGACT T) (Lane 1991); 2.0 µL of 2.5 mM dNTPs; 1.87 µLof 25 mM $MgCl_2$; 2.5 µL of Taq buffer; and 0.3 µL of 5U of Taq DNA polymerase enzyme (Fermentas); 0.05 µL (10 mg mL^{-1}) of bovine serum albumin (BSA), with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 3 min at 95 °C; 30 cycles of 20 s at 94 °C; 40 s at 53 °C; 40 s at 72 °C and 7 min at 72 °C. The 2nd PCR was prepared for a final volume of 50 µL containing: 1.0 µL of the product of the 1st PCR; 0.2 µL (0.2 µM) of each *primer* [F968-GC (5′-AACGCGAAGAACCT TAC-3′)] (Nübel et al. 1996) and R1387 (5′-GCCCGGGAACGTATTCACCG-3′) (Heuer et al. 1997); 4.0 µL 2.5 mM dNTPs; 5.0 µL 25 mM $MgCl_2$; 5.0 µL 10 × Taq buffer; 0.5 µL formamide; and 0.5 µL of the Taq DNA polymerase enzyme (Fermentas), with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 4 min at 94 °C; 30 cycles of 1 min at 94 °C; 1 min at 56 °C; 2 min at 72 °C and 10 min at 72 °C.

NifH Gene

The 1st PCR for the amplification of the *nif*H gene utilized the *primers* [FGPH19 (5′-TACGGCAARGGTGGNATH-3′)] (Simonet et al. 1991) and PolR (5′-ATSGCCATCATYTCR CCG-3′) (Poly et al. 2001). Amplification occurred in 25 µL of the final volume, containing: 2.0 µL 2.5 mM dNTP; 2.5 µL of Taq Buffer (Fermentas); 2.5 µL of 25 mM $MgCl_2$; 0.05 µL (10 mg mL-1) BSA; 5.0 µL of 5U µL-1 of Taq DNA Polymerase (Fermantas); and 0.125 µL of each *primer*

(10 pmol μL^{-1}); 2.0 µL of total DNA (10 ng) of rhizospheric soil or roots, with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 5 min at 94 °C; 30 cycles of 1 min at 94 °C; 1 min at 56 °C; 2 min at 72 °C and 30 min at 72 °C.

In the 2nd PCR, amplification occurred in 50 µL of the final volume, containing 2.0 µL of the product of the amplification of the 1st PCR; 0.250 µL (10 pmol μL^{-1}) of each *primer* (PolF-GC (5′- CGCCCGCCGCGCCCCGCGCCC GGCCCGCCGCCCCCGCCCCTGCGAYCCSAARGCB-GACTC-3′) and AQER (5′-ACGATGTAGATYTCCTG-3′); 4.0 µL 2.5 mM dNTP; 5.0 µL of Taq Buffer (Fermentas) (Poly et al. 2001); 2.0 µL of 25 mM $MgCl_2$; 0.2 µL of 5U µL-1 of Taq DNA Polymerase (Fermantas), with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled under the following cycle conditions: 5 min at 94 °C; 30 cycles of 1 min at 94 °C; 1 min at 56 °C; 2 min at 72 °C and 30 min at 72 °C.

All PCR products were verified in 1% agarose gel in 1 × TAE to confirm the amplification of the desired product.

DGGE analyzes were performed utilizing the Ingeny PhorU System (Ingeny, Goes, The Netherlands) with 6% (w/v) polyacrylamide gels and a denaturing gradient of 45–65% for the PCR product of the 16S rRNA gene and 40–65% for the *nif*H gene.

The gels were electrophoresed at 60 °C for 16 h at 100 and 75 V for the 16S rRNA and *nif*H genes respectively. After electrophoresis, the gels were stained with SYBR-gold (Invitrogen, Breda, The Netherlands) and 1 × TAE in a ratio of 1: 10,000 for 30 min. and photodocumented.

Cultivable bacterial community

Bacterial isolates

The bacterial isolates were obtained following analytical procedures suggested by Dobereiner et al. (1995) utilizing NFb semi-solid medium, N-free and selective for N-fixing bacteria. The bacterial isolates were incubated at 28 °C for eight days and then reinserted in NFb semi-solid medium.

Root samples were washed in running water to remove residual soil and isolate the bacteria. To disinfect the roots, approximately 3 g were immersed in 70% alcohol for 1 min and sodium hypochlorite (≈ 2–2.5% of active chlorine) for 3 min, and again submerged in 70% alcohol for 30 s, and were rinsed twice in sterile distilled water.

Root samples were cut into small segments and ground into 10 mL of phosphate buffered saline (PBS) (1.44 g L^{-1} of Na_2HPO_4; 0.20 g L^{-1} of KCL; 8 g L^{-1} of NaCl; in pH 7.4).

The bacteria from the rhizosphere were isolated weighing 5 g of rhizospheric soil and placed in vials containing 5 g of glass beads and 25 ml of PBS buffer. Both suspensions from rhizosphere and roots were shaken on a horizontal table under of 3.387 g rotation, at 28 °C for 40 min.

Subsequently, 100 µL of serial dilutions (10–4, 10–5 and 10–6) were inoculated in triplicates in NFb semi-solid medium [5 g L^{-1} of malic acid; 0.5 g L^{-1} of K_2HPO_4; 0.2 g L^{-1} of $MgSO_4$. $7H_20$; 0.1 g L^{-1} NaCl; 0.01 g L^{-1} of $CaCl_2$. $2H_2O$; 4 mL L^{-1} of FeEDTA (1.64% solution); 2 mL L^{-1} of bromothymol blue (0.5%); 2 mL L^{-1} of micronutrient solution (0.2 g L^{-1} of $NaMoO_4.2H_2O$ 0.235 g L^{-1} of $MnSO_4$. H_2O; 0.28 g L^{-1} of H_3BO_3, 0.008 g L^{-1} of $CuSO_4.5H_2O$); 175 g L^{-1} of Agar; in pH 6.8], combined with 50 µg mL^{-1} of the fungicide Cercobin 700 (Thiophanate Methyl).

All inoculates of the NFb medium were re-inoculated at 28 °C for an additional 8 days until a growth halo formed inside the medium. After the first re-inoculated, two more successive re-inoculated were made, transferring 100 µL of each culture sample to a new NFb semi-solid medium.

Purification of the colonies was performed by streaking technique in solid NFb medium combined with yeast extract (20 mg L^{-1}). The bacterial isolates at this stage were selected according to differences in morphological characteristics, stored in liquid *Tripase Soy Agar* (TSA) medium plus 20% glycerol and maintained at -20 °C.

Bacterial population density (BPD)

BPD was estimated by the most probable number method (PNM) according to McCrady's classification (Dobereiner et al. 1995). BPD was expressed per gram of fresh plant tissue (FPT) or rhizospheric soil (RS) (colony forming unit—CFU g^{-1} FPT or RS).

Plant growth-promoting

The indicators of plant growth-promoting of the bacterial isolates was tested "in vitro" (present positive control) for: BNF, PS, QS and IAA production.

The identification of potential N-fixing bacteria was performed according to Doberainer et al. (1995). Thus, 100 µL of the bacterial culture was inoculated into semi-solid medium selective (NFb) and incubated for 8 days at 28 °C. The positive result for BNF was identified by the horizontal halo formation of bacterial growth within the culture medium. Tests were performed in triplicate and the experiment was repeated twice to verify the results, which was confirmed by bacterial identification through the genetic sequencing partial of the 16S rRNA gene.

To evaluate the phosphate production potential of the bacterial community, bacteria were inoculated in TSA solid culture medium containing insoluble Ca phosphate (5 g L^{-1} of NH_4Cl; 1 g L^{-1} of $MgSO_4.7H_2O$; 4 g L^{-1} of $CaHPO_4$; 15 g L^{-1} of Agar) buffered at pH 7.2 and

maintained in incubation for 72 h at 28 °C (Tikoo et al. 2001). This procedure was performed in triplicate. The formation of a clear halo around the colonies indicated PS. The ratio between the diameter of the halo solubilization (Ø halo) and the diameter of the corresponding bacterial colony (Ø colony) was used to calculate the solubilization index (SI), according to the expression: [SI = Ø halo (mm)/Ø colony (mm)] (Berraqueiro et al. 1976).

The selection of QS type N-acyl homoserine lactones (AHL) producing bacteria was performed by bioassay using the bacterium *Agrobacterium tumefaciens* NTL4 (pZLR4), AHL biosensor (Steindler and Venturi 2007). *Agrobacterium tumefaciens* was linearly inoculated at the edge of petri dishes containing Luria Bertani medium (LB), combined with 10 µg mL^{-1} of X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto- pyranoside) over the entire surface. Bacterial isolates were inoculated transversely to *Agrobacterium tumefaciens* and the slides were incubated for 48 h at 28 °C. The *Agrobacterium tumefaciens* biosensor strain contains the TraR promoter (fusion of the TraG:: LacZ gene), which regulates the expression of the *LacZ* operon. The QS AHLs molecules bind to the TraR promoter and activate *LacZ* gene expression, resulting in the encoding of the β-galactosidase enzyme, which breaks down the X-gal molecule, turning the cell blue (Steindler and Venturi 2007). Therefore, the observation of *Agrobacterium tumefaciens* colonies with blue pigmentation indicated the production of AHLs by the bacterial isolates evaluated, indicating the potential for microbial biofilm formation.

The capacity of bacterial isolates to produce IAA was evaluated by means of a specific colorimetric method to quantify phytohormone production (Crozier et al. 1988). Isolated colonies were inoculated in TSA 10% [(1.5 g L^{-1} of tryptone; 0.5 g L^{-1} of soy peptone; 1.5 g of NaCl; buffered to pH 7.3)] and maintained in constant agitation for 24 h. Subsequently, 10 µL of this inoculum was again inoculated in liquid TSA medium supplemented with 5 mM L-tryptophan. This procedure was repeated three times. After 24 h of constant agitation (4.61 g), 2 mL of the bacterial culture was centrifuged for 5 min at 0.887 g and then 1400 µL of the supernatant was combined with 600 µL of the Salkowski reagent (2% of 0.5 M $FeCl_3$ in 35% of perchloric acid), incubated without light for 30 min at 28 °C. The positive result for the detection of IAA was characterized by the expression of a pink coloration, which was mensured by spectrophotometer at wavelength of 530 nm. IAA concentration was estimated using a standard curve using known IAA values (0, 50, 100, 150, 200, 250, 300 and 350 µg mL^{-1}) in an not inoculated sterile culture medium (Araújo and Guerreiro 2010).

Genetic diversity: BOX-PCR

Genomic DNA from the bacteria was extracted using the Genomic DNA Purification Kit (Fermentas).

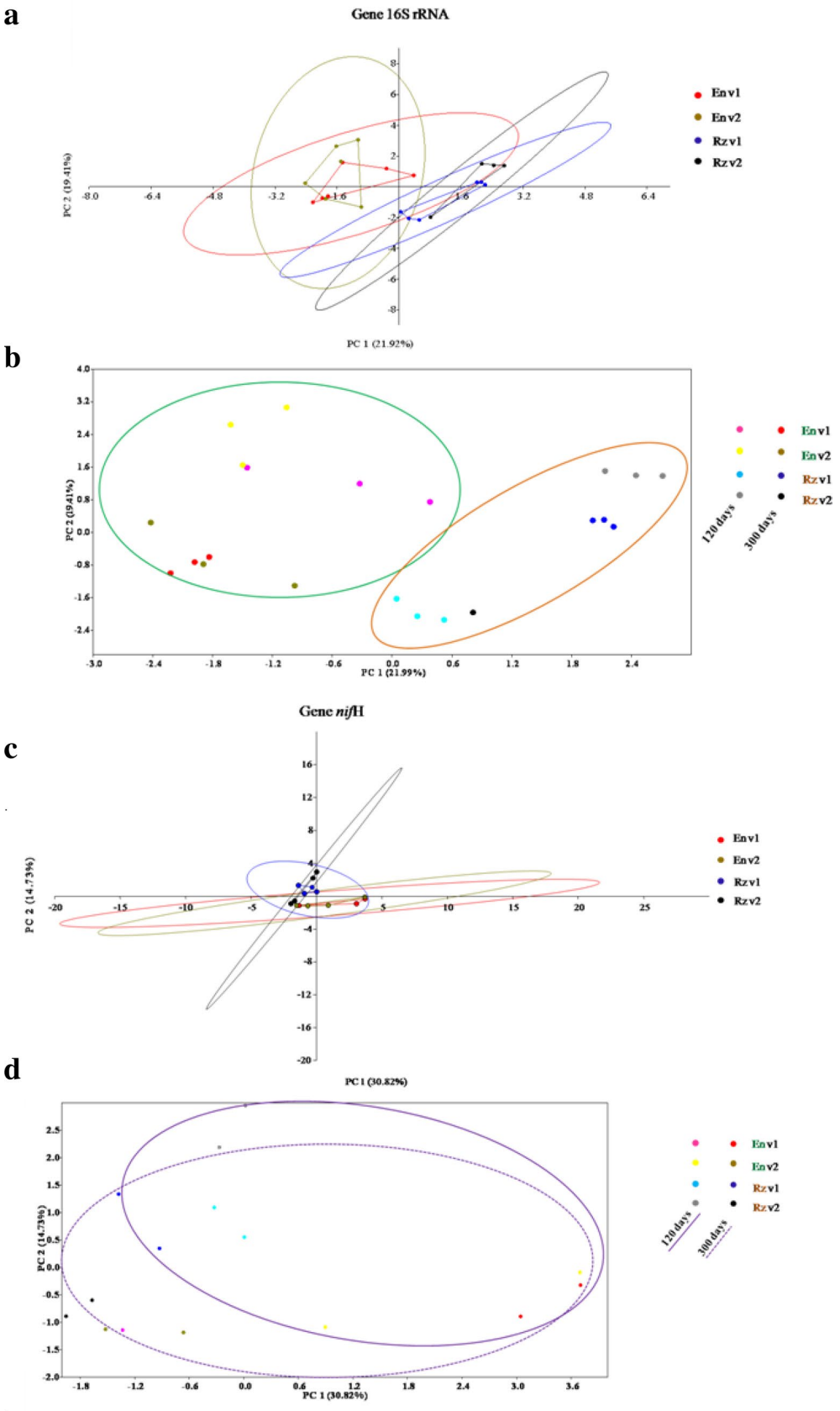
PCR was performed with the first BOX-A1R (5′-CTA CGGCAAGGCGACGCTGACG-3′) (Versalovic et al. 1994) in a final volume of 25 µL, containing: 1.0 µL template DNA (10 ng quantified via Nanodrop spectrophotometer), DNA stock diluted into ultrapure water; 0.1 µL (1 µM) of primer; 10 µL of each 1 mM dNTPs; 2.5 µL of 1 × DMSO (dimethylsufoxamide); 2.5 µL of 1 × *Taq Buffer* enzyme; 3.5 µL of 3.5 mM $MgCl_2$; and 0.4 µL of Taq DNA polymerase (Fermentas) (0.08 U), with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled, under the following cycle conditions: 2 min at 95 °C, 35 cycles of 2 min at 94 °C, 30 s at 92 °C, 1 min at 50 °C and 10 min at 65 °C.

After amplification, the reaction was evaluated by agarose gel electrophoresis (1.5% w/v) for approximately 4 h in 1 × TAE buffer (40 mM Tris–acetate; 1 mM EDTA). The marker utilized was 1 Kb, stained with *Blue green loading dye* (LGC Bio), which was observed under ultraviolet light and photodocumented.

Bacterial isolates identification

The selection of DNA sequencing isolates for bacteria identification was performed considering the similarity of the groups, according to BOX-PCR similarity matrix groupings. Bacterial isolates were selected from groups of higher and lower similarity. Therefore, sampling represented all isolates and avoided repeatability. Twenty-six bacterial isolates were selected at 120 DAR, been 12 root endophytic and 14 rhizospheric; sixteen bacterial isolates were selected at 300 DAR, been 13 root endophytic and 3 rhizospheric.

The amplification of the 16S rRNA was performed by PCR using universal primers [P027F bacteria (5′-GAGAGT TTGGCCTGGCTCAG-3′)] and 1492R (5′-GGTTACCTT GTTACGACTT-3′) (Lane 1991; Zehr and Turner 2001). Reactions were prepared to a final volume of 50 µL, containing: 1.0 µL template DNA (0.5–10 ng); 0.1 µL (0.2 µM) of each primer; 4.0 µL of each 0.2 mM dNTPs; 7.5 µL of 3.75 mM $MgCl_2$; 0.5 µL Taq DNA polymerase (*Fermentas*) (0.05 U); and 5.0 µL of 10 × Taq Buffer, with ultra-pure autoclaved water accounting for the remaining volume. The reaction was thermocycled, under the following cycle conditions: 4 m at 94 °C, 25 cycles of 30 s at 94 °C, 1 min at 63 °C and 1 min at 72 °C. After amplification, 5 µL of the PCR were evaluated by agarose gel electrophoresis (1.2% w/v) in 1 × TAE buffer, GeneRuler 100 bp DNA Ladder marker (Thermo Scientific) and then observed under ultraviolet light and photodocumented.


a
Gene 16S rRNA
PC 2 (19.41%)
PC 1 (21.92%)
Env1
Env2
Rzv1
Rzv2
b
PC 2 (19.41%)
PC 1 (21.99%)
120 days
300 days
Gene nifH
c
PC 2 (14.73%)
PC 1 (30.82%)
d
PC 2 (14.73%)
PC 1 (30.82%)

◂**Fig. 1** Principal component (PC) analysis of the total bacterial community determined through *Denaturing Gradient Gel Eletrophoresis* (DGGE) of the 16S rRNA and *nif*H gene, respectively, in different habitats of the plant/bacteria association in sugarcane in the varieties RB 92579 e RB 867515, independent of the phonological phase of the crop (**a** and **c**); and PC analysis of the total bacterial community determined through DGGE of the 16S rRNA and *nif*H gene, respectively, in different habitats of the plant/bacteria association in sugarcane in the varieties RB 92579 e RB 867515 at 120 and 300 days after regrowth (DAR) (**b** and **d**); root endophytic habitat (En); rhizospheric soil habitat (Rz); RB 92579 variety (v1); and RB 867515 variety (v2)

For the identification of the isolates, 16S rDNA PCR products were purified (ChargeSwitch® Nucleic Acid Purification Technology) and subjected to partial 16S rRNA sequencing with the 1492R primer. The sequences were analyzed by BLASTn, using the National Center for Biotechnology Information website (NCBI) (https://www.ncbi.nlm.nih.gov). The sequences were deposited in the NCBI database (GenBank).

Eight bacteria from this bank were used in the study by Lima et al. (2018) who evaluated the tolerance of these bacteria to adverse environmental conditions. The study also evaluated under controlled conditions some characteristics of plant growth-promoting of these bacteria.

Statistical procedures

Genetic diversity was evaluated through the analysis of agarose and polyacrylamide gels, and the bands observed by the amplification were transformed into binary data, creating a binary matrix or similarity of the evaluated genetic profile. For PCR-DGGE, the profiles obtained for 'amplicons' were analyzed and compared using the 'Image Quant Software' program (Molecular Dynamics, Sunnyvale, CA, USA), generating the binary matrix utilized to evaluate the genetic diversity.

The BOX-PCR matrix was performed manually by observing the presence or absence of bands in the gel, utilizing the principal components analysis and Shannon–Weaver index of similarity.

From the similarity matrix, the diversity of the root endophytic and rhizosphere bacterial community was evaluated for the RB 92579 and RB 967515 varieties at 120 and 300 DAR by principal component analysis and the significance of the separation of the bacterial communities was realized by ANOSIM similarity analysis. ANOSIM analysis allows the identification of significant differences based on algorithms of average distances between groups generating correlation R. $R > 0.75$ indicate that the groups are distinct and separated; $0.25 > R < 0.75$ indicate that groups are distinct but some overlap occurs; and $R < 0.25$ indicate that groups are not distinct and do not separate (Clarke and Gorley 2001).

Population density data was submitted to variance analysis (ANOVA) and analyzed in factorial arrangement, according to varieties and plant bacteria association habitats in each phenological phase of ratoon-cane (120 and 300 DAR). When the main effects and/or interactions were significant, the averages were compared by the Scott-Knott test ($P < 0.05$).

The indicators of plant growth-promotion in the cultivable bacterial community was evaluated through a qualitative analysis, evaluating the amount of bacterial isolates capable of expressing one or more of the plant growth-promotion indicators (BNF, PS, QS and IAA production) and the amount of isolates that failed to exhibit one or more of these indicators. Thus, we were able to calculate the relative frequency, which was obtained by the ratio between the total number of bacterial isolates and the number of isolates capable of plant growth-promoting.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.74160599 is shown. The percentage of replicate trees in which the associated taxons clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are shown in the units of the number of base substitutions per site. The analysis involved 36 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 768 positions in the final dataset. Evolutionary analyses were conducted via MEGA6.

Results

Genetic diversity of the total bacterial community

Analysis of the bacterial community by DGGE of the 16S rRNA gene (Fig. 1a, b) and the *nif*H gene (Fig. 1c, d) indicated a high genetic diversity in the root endophytic and rhizospheric soil habitats of RB 92579 and RB 867515 varieties.

The total bacterial community determined by DGGE of the 16S rRNA gene differed in two large groups according to association habitat, variety and phenological phase of the plant (Fig. 1a). There was a clustering of overlapping bacterial community in the root endophytic habitat at 120 DAR, which did not persist at 300 DAR (Fig. 1b). Therefore, in general, there was a significant change in the structure of the bacterial community in the different habitats, varieties and

phenological phase, as can be confirmed by the ANOSIM similarity analysis (Table 1).

The analysis of the *nif*H gene for the total bacterial community identified the formation of subdivisions from in the root endophytic and rhizospheric soil habitats, formation dominant groups (Fig. 1c, d), which was then verified by ANOSIM similarity analysis (Table 2). Analysis of the *nif*H gene in the root endophytic habitat found that there were no distinct groups at 120 or 300 DAR, even in different sugarcane varieties (Table 2). In this case, the structure of the

Table 1 *R* values[a] of the analysis of similarity of the total bacterial community determined through *Denaturing Gradient Gel Electrophoresis* (DGGE) of the gene 16S rRNA in different habitats of the plant/bacteria association in sugarcane in the second crop cycle (cane-ratoon) in the RB 92579 and RB 867515 varieties to 120 and 300 days after regrowth (DAR)

Variety/DAR	Root endophytic			
	RB 92579-120	RB 92579-300	RB 867515-120	RB 867515-300
RB 92579-120	–	–	–	–
RB 92579-300	0.926	–	–	–
RB 867515-120	0.593	0.889	–	–
RB 867515-300	1.000	0.815	0.899	–
	Rhizospheric soil			
	RB 92579-120	RB 92579-300	RB 867515-120	RB 867515-300
RB 92579-120	–	–	–	–
RB 92579-300	1.000	–	–	–
RB 867515-120	1.000	1.000	–	–
RB 867515-300	1.000	1.000	1.000	–

[a]$R > 0.75$ indicates that the groups are distinct and separate; $0.25 > R < 0.75$ indicates that the groups are distinct but present overlaps; and $R < 0.25$ indicates that the groups are not distinct and do not separate (Clarke and Gorley 2001)

Table 2 *R* values[a] of the analysis of similarity of the total bacterial community determined through *Denaturing Gradient Gel Electrophoresis* (DGGE) of the gene *nif*H in different habitats of the plant/bacteria association in sugarcane in the second crop cycle (cane-ratoon) in the RB 92579 and RB 867515 varieties to 120 and 300 days after regrowth (DAR)

Variety/DAR	Root endophytic			
	RB 92579-120	RB 92579-300	RB 867515-120	RB 867515-300
RB 92579-120	–	–	–	–
RB 92579-300	1.000	–	–	–
RB 867515-120	1.000	0.000	–	–
RB 867515-300	0.500	1.000	1.000	–
	Rhizospheric soil			
	RB 92579-120	RB 92579-300	RB 867515-120	RB 867515-300
RB 92579-120	–	–	–	–
RB 92579-300	1.000	–	–	–
RB 867515-120	0.500	0.500	–	–
RB 867515-300	1.000	1.000	1.000	–

[a]$R > 0.75$ indicates that the groups are distinct and separate; $0.25 > R < 0.75$ indicates that the groups are distinct but present overlaps; and, $R < 0.25$ indicate that the groups are not distinct and do not separate (Clarke and Gorley 2001)

bacterial community did not change with phenological phase or with different varieties.

Cultivable bacterial community

Population density

One hundred twenty three bacteria associated with sugarcane plants were isolated. In plants at 120 DAR, 31 root endophytic bacteria and 46 rhizospheric soil bacteria were isolated, and in plants at 300 DAR we isolated 26 root endophytic bacteria from the roots and 20 bacteria from the rhizospheric soil.

Bacterial population density did not differ according to sugarcane variety or phenological phase of the plant. The density of bacteria in rhizospheric soil habitat was higher of than in the root endophytic habitat in RB 867,515 at 120 DAR (Fig. 2).

Plant growth-promotion

Total bacterial isolates at 120 DAR presented potential for BNF and IAA production, independent of variety and habitat (Fig. 3a, b). Bacterial isolates' ability to solubilize phosphate varied according to association habitat and was dependent on sugarcane variety. Relative frequency shown as all bacterial isolates from the root endophytic habitat of RB 867515 solubilized phosphate (Fig. 3a) and all bacterial isolates from the rhizospheric soil habitat of RB 92579 solubilized phosphate (Fig. 3b). The potential to bind atmospheric N_2 was the most strongly identified plant growth-promoting property in bacterial isolates (Fig. 3).

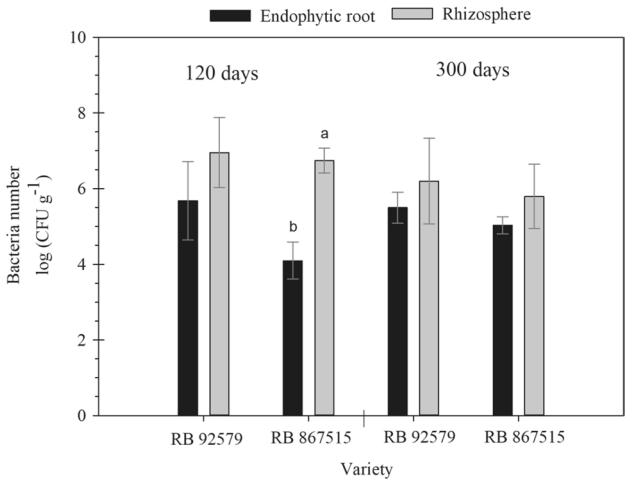


Fig. 2 Population density of the cultivated bacterial community in the habitats root endophytic and rhizospheric soil of the varieties RB 92579 e RB 867515 sugarcane at 120 and 300 days after regrowth (DAR). Means followed by the same letter in the column did not differ by the Scott-Knott test ($p < 0.05$)

The plant growth-promotion properties were reduced at 300 DAR in both habitats, independent of sugarcane variety. BNF potential continued to occur at 300 DAR in 100 and 98% of the isolates of RB 867515 and RB 92579 varieties, respectively (Fig. 3c, d).

QS production was more expressive at 300 DAR in the root endophytic habitat of RB 867515 (Fig. 3c).

Genetic diversity: BOX-PCR

Bacterial genetic diversity was evaluated by BOX-PCR in 77 bacterial isolates at 120 DAR and 32 isolates at 300 DAR in the root endophytic and rhizosphere habitats of sugarcane. It was possible to visualize bands between 100 and 2000 pb generated by the use of the BOX-A1R *primer* that amplified repetitive sequences of bacterial genomic DNA. The data suggests high bacterial diversity and low similarity between isolates, regardless of association habitat and variety (Fig. 4a, b). The Shannon–Weaver diversity index of the root endophytic bacterial community did not differ from the rhizospheric bacterial community, regardless of the variety and phenological stage of sugarcane (Table 3).

Cultivable bacterial community identification

According to the partial sequencing of the 16S rRNA gene, the presence of different bacterial genera differed according to the plant/bacteria association habitat, sugarcane variety and phenological phase (Table 4).

In general, both habitats presented high genetic diversity, with *Burkholderia* sp., *Enterobacter* sp., *Pseudomonas* sp., *Pantoea* sp. and *Stenotrophomonas* sp. (Fig. 5). The genera *Bacillus* sp. and *Dyella* sp. were restricted to roots in the variety RB 92579, showing a greater specificity in relation to habitat and variety (Fig. 5).

We observed at 120 DAR a high genetic diversity in the root endophytic habitat of the RB 92579 variety, which was reduced in the rhizospheric soil habitat. There was at 300 DAR reduced genetic diversity in both habitats and varieties, with the exception of one bacterium present in the RB 92579 variety in the rhizospheric soil habitat. Therefore, the RB 92579 variety presented higher colonization of bacteria in the root endophytic habitat and RB 867515 in both habitats (Fig. 5).

Phylogenetic analysis showed the presence of proteobactea (97.23%) and firmicutes (2.78%), with the genus *Bacillus* sp. found endophytically in the roots of sugarcane (Fig. 6). The proteobacteria group was subdivided into alpha-proteobacteria (γ-Proteobacteria) and beta-proteobacteria (β-*Proteobacteria*), with a relative frequency of 80.56% and 16.67%, respectively (Fig. 6). The bacteria of the phylum-Proteobacteria were of the genus *Enterobacter* sp., *Pantoea* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Dyella* sp.

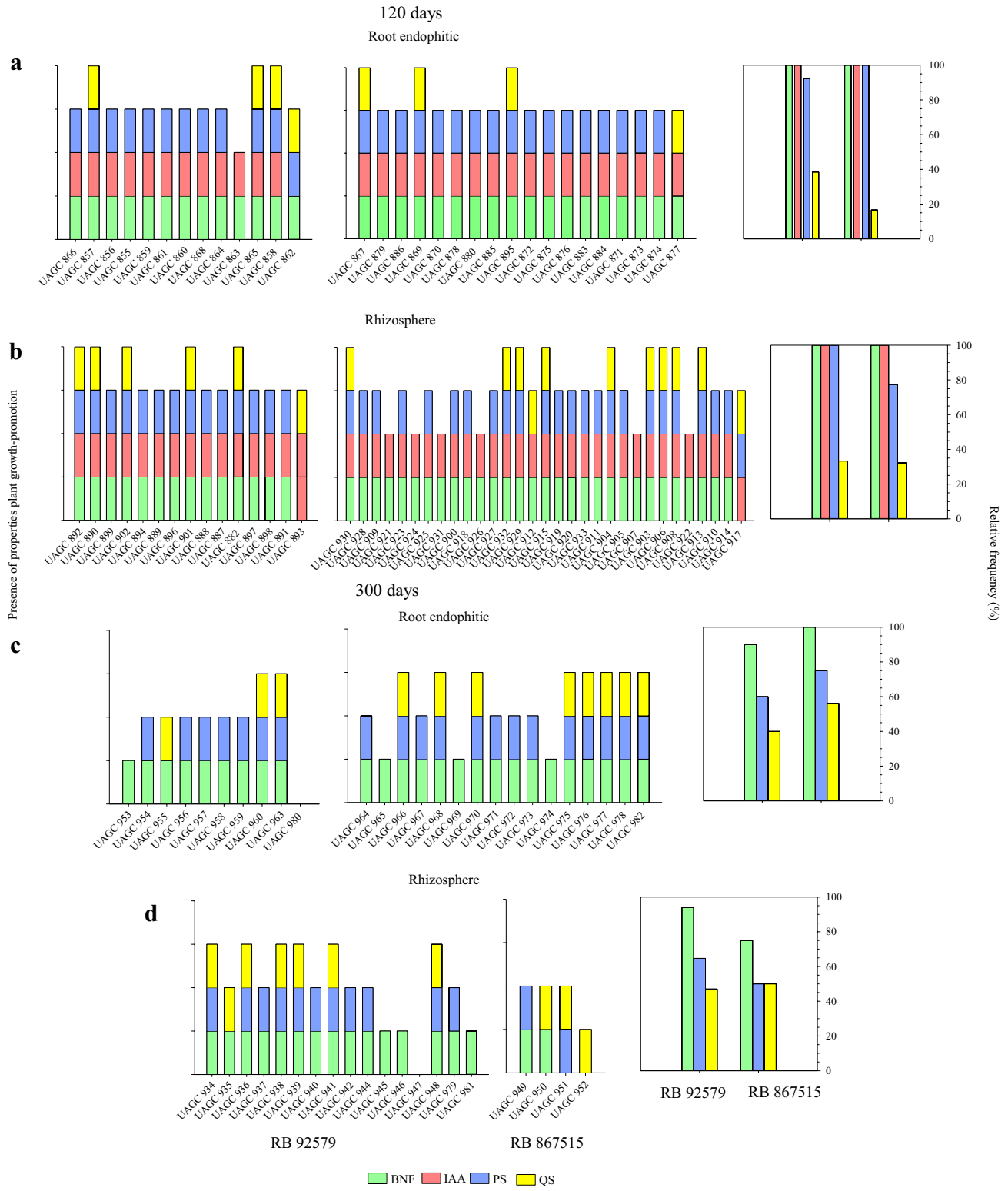


Fig. 3 Plant growth-promotion of the bacteria root endophitic (**a**) and rhizosphere (**b**) at 120 days after regrowth (DAR) in the varieties RB 92579 e RB 867515; plant growth-promotion of the bacteria root endophitic (**c**) and rhizosphere (**d**) at 300 DAR in the varieties RB 92579 e RB 867515 with the respective relative frequency. Biological N fixation (BNF); indole acetic acid (IAA) production; phosphate solubilization (PS); *quorum-sensing* (QS). More information in the supplementary material A1 and A2

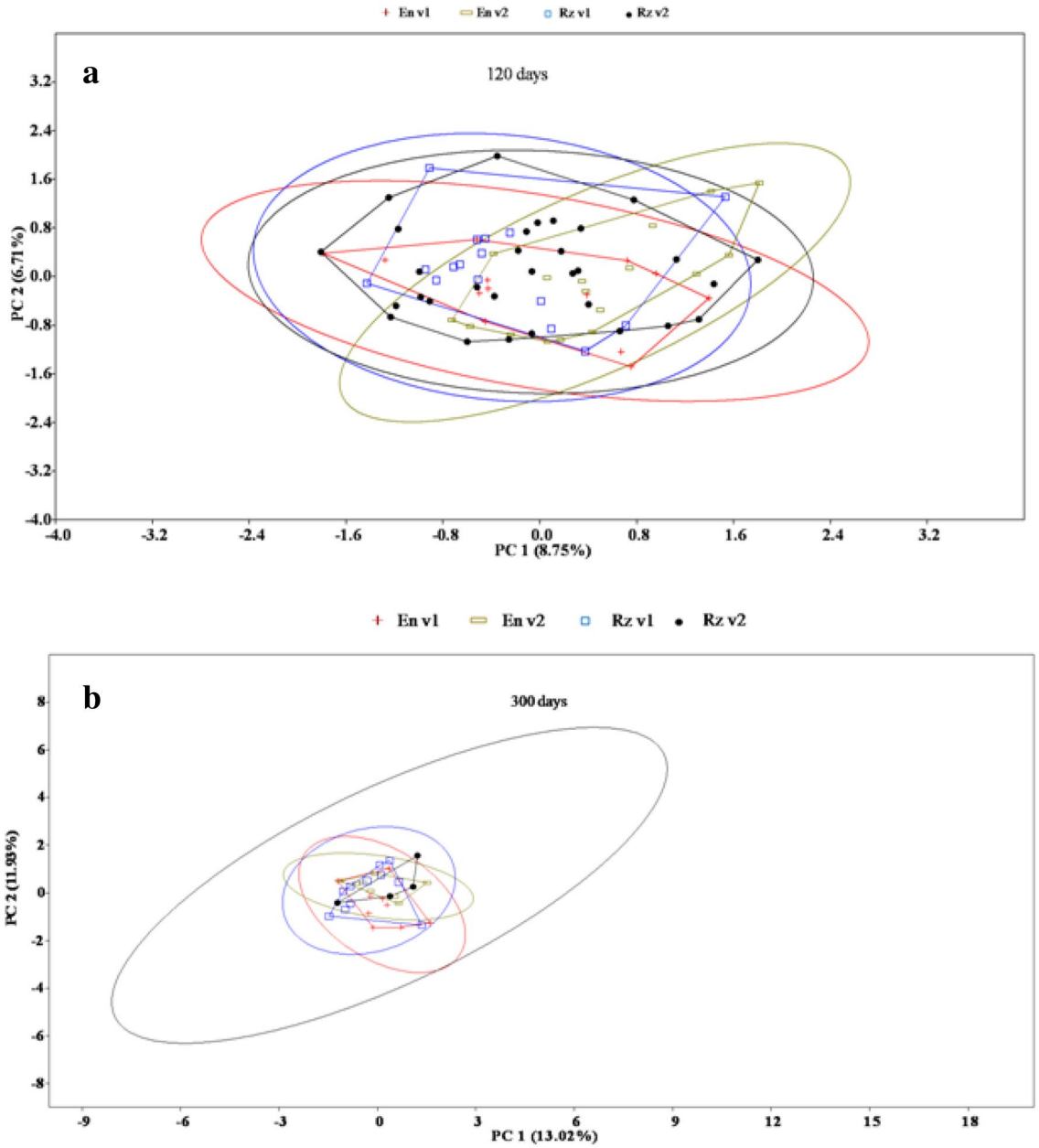


Fig. 4 Principal component (PC) analysis of the cultivated bacterial community determined through *Polymerase Chain Reaction* (BOX-PCR) in the root endophitic and rhizospheric soil habitat of the sugarcane/bacteria association at 120 (**a**) and 300 (**b**) days after regrowth (DAR), respectively, in the varieties RB 92579 and RB 867515. Root endophytic habitat (En); rhizospheric soil habitat (Rz); variety RB 92579 (v1); and variety RB 867515 (v2)

and the phylum *β-Proteobacteria* was found only the genus *Burkholderia* sp.

Discussion

High genetic diversity was observed in this study for both the total and cultivable potentially N-fixing bacterial community utilizing the DGGE and BOX-PCR techniques, respectively. Our study also identified some isolates of the bacterial community associated with sugarcane and demonstrated this high genetic diversity.

Supriadi et al. (2020) studying genetic variability of root colonizing bacteria and rhizospheric soil in sugarcane reported that a total of 1259 operational taxonomic units (OTUs) were detected in root bacteria and 3894 OTUs in soil bacteria. The authors reported that the diversity between soil and root bacteria was significantly different.

Table 3 Shannon–Weaver diversity índex of the bacterial community root endophytic and rhizospheric soil in sugarcane of the varieties RB 92579 and RB 867515 at 120 and 300 days after regrowth (DAR)

Variety	Association habitat		Mean
	Root endophytic	Rhizospheric soil	
120 DAR			
RB 92579	2.39	2.40	2.39
RB 867515	2.56	2.48	2.52
Mean	2.47	2.44	
	F teste ($p < 0.05$)		
Association habitat	0.28 ^{ns}		
Sugarcane variety	0.06 ^{ns}		
Habitat × variety	0.48 ^{ns}		
CV (%)[a]	10.92		
300 DAR			
RB 92579	2.22	2.01	2.11
RB 867515	1.98	2.27	2.12
Mean	2.10	2.14	
	F teste ($p < 0.05$)		
Association habitat	0.07 ^{ns}		
Sugarcane variety	0.00 ^{ns}		
Habitat × variety	2.94 ^{ns}		
CV (%)	18.17		

[a]CV, coefficient of variation = standard deviation/mean × 100; *ns* not significant

In our study, we showed that this diversity was reduced at the end of the sugarcane cultivation cycle in both habitats (root endophytic and rhizospheric soil). Thus, diversity was greater at the beginning of the cycle, favoring the growth of plants due to the importance of these bacteria as promoters of plant growth and N-fixers. This greater diversity can also be associated with environmental adversity. For example, Lamizadeh et al. (2019) showed that saline soils cultivated with sugarcane showed greater genetic diversity when compared to non-saline soils; however, the microbial biomass and respiration rate was lower with salinity. The different ecosystems existing in the biosphere have habitats with high microbial diversity, since the microorganisms have different functions in the natural and agricultural ecosystems, ensuring the balance of the system (Ho et al. 2017).

To evaluate the genetic and functional diversity of the microorganisms present in the soil/plant interaction, DGGE is also used to evaluate the environmental variation of the bacterial community under the influence of biotic and abiotic factors (Yeoh et al. 2016). There are few studies that seek to study ecological interactions and identify potentially N-fixing bacteria in commercial crops of sugarcane plants in Northeast Brazil. DGGE analysis in this study indicated that the potentially N-fixing total bacterial community showed high genetic diversity, independent of habitat, variety and phenological phase of sugarcane. Similar results were found by Jorquera et al. (2012), evaluating the genetic diversity of the rhizospheric bacterial communities of *Larrea tridentata* (Sesse and Moc. Ex DC) Coville using DGGE with the 16S rRNA gene, observed high diversity in desert environments. Yeoh et al. (2016) evaluated the bacterial diversity in roots and rhizospheric soil of sugarcane with the 16S rRNA and *nif*H gene by DGGE also observing high genetic variability with moderate use of N fertilizer.

The *nif* genes are necessary in several species of bacteria for the synthesis and functioning of the nitrogenase enzyme that acts on BNF (in this case, *nif*H) to transform the atmospheric N_2 into NH_3, making the N assimilable by the plants (Zhan and Sun 2012). The authors observed structural differences in the communities of these microorganisms from *amplicon* profiles after DGGE of the *nif*H gene, evidencing the genetic diversity of the bacteria populations. Having high genetic diversity is of great importance, as it increases the possibility of finding N-fixing bacteria, adapted to local reality and efficiently plant growth-promoting that can be used as biostimulants (Jardin 2015). Our study also used the *nif*H gene and the DGGE of this gene finding a high diversity of the total N-fixing bacterial community.

The root endophytic habitat is a more favorable environment for microorganisms, because offers shelter with greater stability and have carbon source readily available. The rhizosphere is a more vulnerable habitat to climatic variations, abiotic and abiotic stresses, competition and food reduction (Backer et al. 2018). Due to these fluctuations and the influence of these characteristics under the bacteria, this study showed a greater bacterial genetic diversity in the root endophytic habitat and a reduction in the rhizosphere.

The reduction of genetic diversity in the rhizospheric habitat was influenced in both periods of collection. Sugarcane in the region is grown under rainfed conditions. There are bacterial groups that do not tolerate or are more sensitive to water deficits, even for short periods. The variation in bacterial genetic diversity in both habitats evaluated was more sensitive and perceptible in the variety RB 92579, indicating less stability in bacterial interaction.

The diversity and density of microorganism that colonize plant roots influence the symbiotic process and show high variation of beneficial responses of plant/bacterial interaction (Wissuwa et al. 2009).

In the case of sugarcane plants, there are many reports of N-fixing bacteria that, in addition to BNF, contribute effectively to the plant growth- promotion (Urquiaga et al. 2012). The BNF capacity in this study was observed "in vitro" in different bacterial isolates at the end of the sugarcane cycle during the phenological phase of maturation and sugar concentration. Depending on the intensity of N-fixation in the plant, the increased N concentration in

Table 4 Identification of bacteria, similarity, GenBank identification, sugarcane variety, association habitat and days after regrowth (DAR) of bacterial isolates

Isolate	Identification	Similarity (%)	GenBank identification	Source		
				Variety	Association niche	DAR
UAGC 863	*Bacillus* sp.	98	KY229201	RB 92579	Root endophytic	120
UAGC 857	*Burkholderia* sp.	98	KY229202	RB 92579	Root endophytic	120
UAGC 895	*Burkholderia* sp.	97	KY229203	RB 92579	Root endophytic	120
UAGC 867	*Burkholderia* sp.	93	KY271631	RB 92579	Root endophytic	120
UAGC 871	*Burkholderia* sp.	97	KY271642	RB 92579	Root endophytic	120
UAGC 866	*Enterobacter* sp.	95	KY229207	RB 92579	Root endophytic	120
UAGC 855	*Pantoea* sp.	97	KY229219	RB 92579	Root endophytic	120
UAGC 858	*Pantoea* sp.	96	KY229220	RB 92579	Root endophytic	120
UAGC 865	*Pantoea* sp.	98	KY229221	RB 92579	Root endophytic	120
UAGC 882	*Pantoea* sp.	96	KY271637	RB 867515	Root endophytic	120
UAGC 905	*Burkholderia* sp.	95	KY271640	RB 867515	Root endophytic	120
UAGC 879	*Enterobacter* sp.	97	KY271632	RB 867515	Root endophytic	120
UAGC 897	*Enterobacter* sp.	97	KY271633	RB 92579	Rhizospheric soil	120
UAGC 901	*Enterobacter* sp.	95	KY229208	RB 92579	Rhizospheric soil	120
UAGC 902	*Pseudomonas* sp.	96	KY229228	RB 92579	Rhizospheric soil	120
UAGC 904	*Burkholderia* sp.	96	KY229204	RB 867515	Rhizospheric soil	120
UAGC 913	*Burkholderia* sp.	89	KY229205	RB 867515	Rhizospheric soil	120
UAGC 903	*Enterobacter* sp.	97	KY229209	RB 867515	Rhizospheric soil	120
UAGC 917	*Enterobacter* sp.	96	KY229210	RB 867515	Rhizospheric soil	120
UAGC 918	*Enterobacter* sp.	96	KY229211	RB 867515	Rhizospheric soil	120
UAGC 929	*Enterobacter* sp.	95	KY229212	RB 867515	Rhizospheric soil	120
UAGC 930	*Enterobacter* sp.	96	KY229213	RB 867515	Rhizospheric soil	120
UAGC 906	*Pantoea* sp.	97	KY229222	RB 867515	Rhizospheric soil	120
UAGC 907	*Pantoea* sp.	97	KY229223	RB 867515	Rhizospheric soil	120
UAGC 908	*Pantoea* sp.	97	KY229224	RB 867515	Rhizospheric soil	120
UAGC 925	*Stenotrophomonas* sp.	89	KY229230	RB 867515	Rhizospheric soil	120
UAGC 980	*Dyella* sp.	97	KY229206	RB 92579	Root endophytic	300
UAGC 955	*Enterobacter* sp.	97	KY229215	RB 92579	Root endophytic	300
UAGC 958	*Enterobacter* sp.	97	KY229216	RB 92579	Root endophytic	300
UAGC 959	*Enterobacter* sp.	96	KY271635	RB 92579	Root endophytic	300
UAGC 963	*Enterobacter* sp.	89	KY229217	RB 92579	Root endophytic	300
UAGC 936	*Enterobacter* sp.	96	KY229214	RB 92579	Rhizospheric soil	300
UAGC 973	*Enterobacter* sp.	96	KY229218	RB 867515	Root endophytic	300
UAGC 972	*Pantoea* sp.	91	KY229225	RB 867515	Root endophytic	300
UAGC 975	*Pantoea* sp.	95	KY271638	RB 867515	Root endophytic	300
UAGC 976	*Pantoea* sp.	95	KY271639	RB 867515	Root endophytic	300
UAGC 977	*Pantoea* sp.	96	KY229226	RB 867515	Root endophytic	300
UAGC 978	*Pantoea* sp.	89	KY229227	RB 867515	Root endophytic	300
UAGC 965	*Stenotrophomonas* sp.	97	KY229231	RB 867515	Root endophytic	300
UAGC 982	*Stenotrophomonas* sp.	96	KY229232	RB 867515	Root endophytic	300
UAGC 950	*Burkholderia* sp.	95	KY271641	RB 867515	Rhizospheric soil	300
UAGC 949	*Pseudomonas* sp.	98	KY229229	RB 867515	Rhizospheric soil	300

this phenological phase may be detrimental to the sugar concentration, because the plant can continue to maintain vegetative growth, reducing the maturation period (Hussain et al. 2017).

Fig. 5 Species name, accession number and gene sequence accession number of the bacteria in the root endophytic and rhizospheric soil habitat in sugarcane varieties RB 92579 and RB 867515 at 120 and 300 days after regrowth (DAR)

In this study were found the following genera: *Burkholderia* sp., *Pantoea* sp., *Bacillus* sp., *Stenotrophomonas* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Dyella* sp. These generous can be potential promising biotechnological for the production of biostimulants, primarily for agriculture due to specificities linked to the ecosystem and variability of the environment. Magnani et al. (2010), evaluating the diversity of the endophytic bacterial community associated with sugarcane, isolated the genera *Pantoea* sp., *Enterobacter* sp., *Klebisiella* sp., *Citrobacter* sp. and *Pseudomonas* sp.

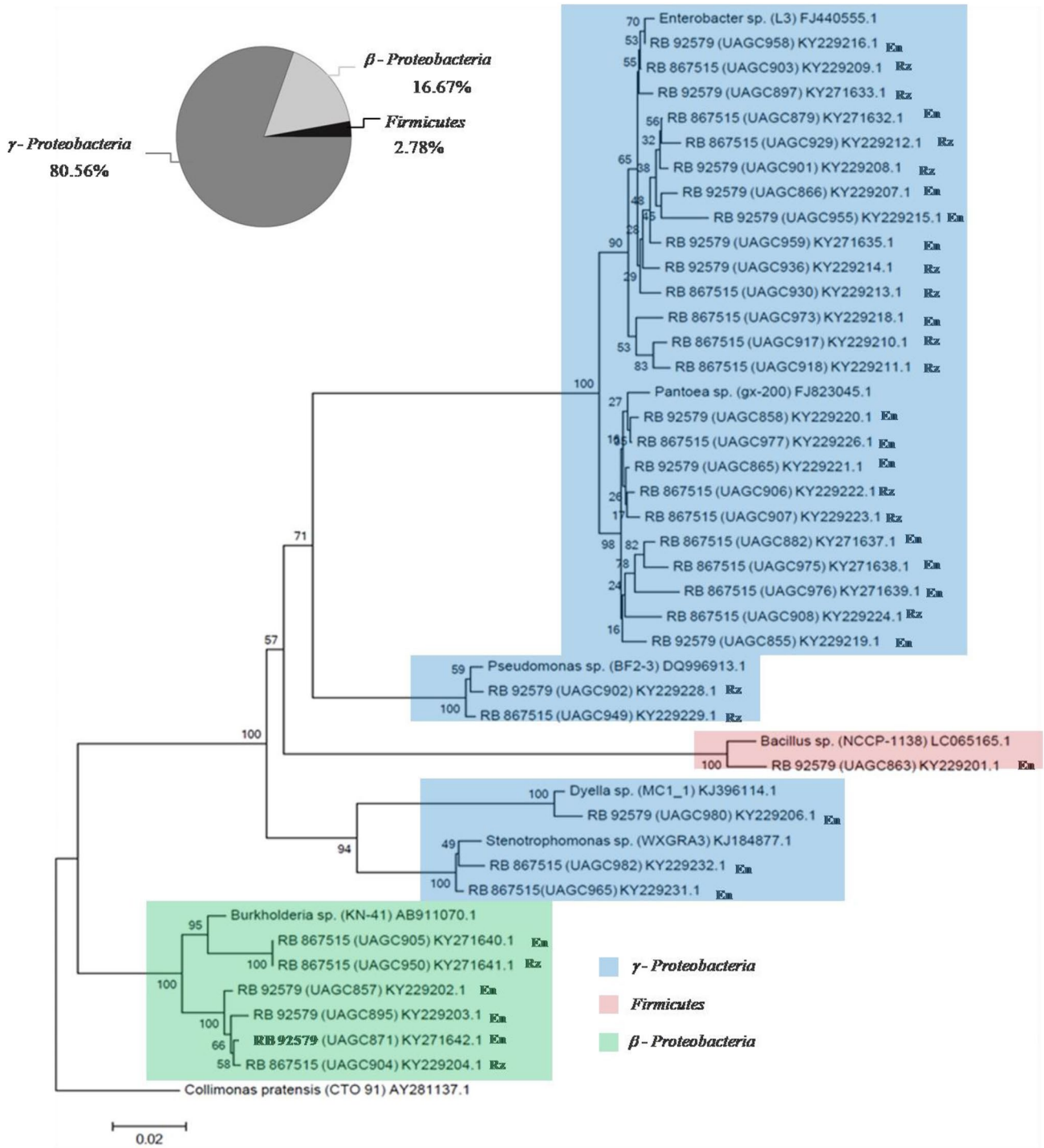


Fig. 6 Phylogenetic tree constructed from the sequences of the 16S rRNA isolated from sugarcane, compared to sequences already deposited in databases, using the Neighbor-Joining method. The values for each branch represent the percentages of 500 bootstrap replicates

Saharan and Nehra (2011) also reported in some studies as sugarcane the bacterial genera: *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., *Herbaspirillum* sp., *Bacillus* sp., *Azospirillum* sp., *Gluconacetobacter* sp. and *Herbaspirillum* sp.

Despite the different bacterial genera found in the ecosystems sugarcane producers, little is known about the diversity, the microbial ecology and the biotechnological potential of the bacteria existing in commercial sugarcane crops in Northeast of the Brazil. This study sought promising

bacterial strains for plants promoting-growth that can be used in the production of biostimulants. Bioprospecting of sugarcane associated bacteria with characteristics of plant growth-promotion potential and the study of these plant/bacteria interaction mechanisms are important for the reduction of the use of N fertilizers in the cultivation of sugarcane.

Conclusions

We found greater genetic diversity in the root endophytic habitat at 120 DAR. Therefore, in general, there was a significant change in the structure of the bacterial community in the different habitats, varieties and phenological phase. The density of bacteria in rhizospheric soil habitat was higher of than in the root endophytic habitat in RB 867515 at 120 DAR. We identify the genera *Burkholderia* sp., *Pantoea* sp., *Erwinia* sp., *Stenotrophomonas* sp., *Enterobacter* sp. and *Pseudomonas* sp. The genera *Bacillus* sp. and *Dyella* sp. were only identified in the variety RB 92579. We found indices above 50% for biological nitrogen fixation, production of indole acetic acid and phosphate solubilization, showing that the use of these bacteria in biotechnological products is very promising.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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